

peptide sequences (e.g., the Src homology-2 or SH2 domain of Src-family kinases bind tightly to a phosphorylated tyrosine (Y\*-EEI) sequence (**SEQ ID NO: 9**) found in epidermal growth factor receptor and the focal adhesion kinase) (61).

Please delete the paragraph on page 21, lines 7-16 and replace it with the following paragraph:

Figure 5 shows a P-glycoprotein predicted secondary structure and amino acid of the linker domain. A schematic representation of P-gp predicted secondary structure. The twelve filled squares represent the twelve putative transmembrane domains. The two ATP binding domains are represented by two circles in the N- and C-terminal halves of P-gp. The inset represents the linker domain. The amino acid sequence of the linker domains of Human P-gp 1 (HP-gp1) and HP-gp3 is indicated as a single-letter amino acid code. The numbers in brackets at the beginning and end of each amino acid sequence of HP-gp1 (**SEQ ID NO: 15**) and HP-gp3 (**residues 1-89 of SEQ ID NO: 14**) shows the length of the linker domains (1 - 90 and 1- 88 for HP-gp1 and HP-gp3, respectively). The numbered lines underneath the amino acid sequence show the sequences of the overlapping hexapeptides, which differ by one amino acid. For HP-gp3, the last hexapeptide is number 88.

Please delete the paragraph on page 41, line 15, through page 42, line 10 and replace it with the following paragraph:

To purify the 57 kDa protein using the two hexapeptides, it was of interest to determine if other carrier proteins than BSA can be used. Figure 12 shows the effects of no blocking carrier, 1% gelatin and 0.3% or 3% BSA on the binding of the hexapeptides to the 57 kDa protein. The results of this experiment were surprising in that no carrier protein was required to reduce the unspecific binding (Figure 12). The latter established binding conditions were used to isolate large amounts of 57 kDa protein that bound to several copies of hexapeptides <sup>658</sup>RSSLIR<sup>663</sup> (SEQ ID NO: 7) and <sup>669</sup>SVRGSQ<sup>674</sup> (SEQ ID NO: 8). Figure 13 shows purified 57 kDa protein on SDS-PAGE stained with Coomassie blue. The latter purified protein was transferred to PVDF membrane and stained with Ponceau S to localize the position of the 57 kDa protein. The Ponceau S-stained band that migrated with the expected molecular mass was cut out and used for direct N-terminal sequencing (33). The first seven rounds of Edman degradation showed two sequences of MREVISI (**SEQ ID**

**NO: 10**) and MREIVHI (**SEQ ID NO: 11**). These two sequences differed only by three amino acids (VIS instead of IVH). Comparison of the two sequence with known protein sequences using FastA protein search engine, showed the latter sequences to encode the first seven N-terminal amino acids of  $\alpha$ - and  $\beta$ -tubulins. The identification of tubulins, as the 57 kDa protein was consistent with the apparent molecular mass and the potential degradation products that were observed following long incubation periods. To further confirm the identity of the 57 kDa protein as tubulins, Western blot analysis was preformed on hexapeptide-bound 57 kDa protein and total cell lysate resolved by SDS-PAGE and transferred to nitrocellulose membrane. The nitrocellulose membrane was then probed with anti  $\alpha$ -tubulin and anti- $\beta$ -tubulin monoclonal antibodies, respectively. Figure 14 shows the results of the Western blot analysis. Consistent with the sequencing results, both tubulin subunits ( $\alpha$  and  $\beta$ ) were recognized in the lanes containing the hexapeptide bound proteins. Thus, establishing the identity of the 57 kDa protein as  $\alpha$  and  $\beta$ -tubulin.

Please delete the paragraph on page 22, lines 14-20 and replace it with the following paragraph:

Figure 10 shows the sequence alignment of three binding regions of HP-gp1 and HP-gp3 linker domains. Alignment of HP-gp1 (**SEQ ID NO: 15**) and HP-gp3 (**SEQ ID NO: 14**) linker domains is shown using a single-letter code for amino acids. The regions of high binding affinities for HP-gp3 and HP-gp1 are shown in bold. Identical amino acids are shown by single letter code between the two aligned sequences. Conserved amino acids are indicated by plus (+) sign. The numbers on each side of the amino acid sequence of the linker domains refer to the amino acid sequence of human P-gp1 and 3 as in (90, 111).

Please delete the paragraph on page 25, lines 4-7 and replace it with the following paragraph:

Figure 15 shows the helical wheel presentations of the high affinity binding region of HP-gp1 and HP-gp3 linker domains. The single-letter amino acid code for the high affinity binding region of HP-gp1 (**SEQ ID NO: 12**) and HP-gp3 (**SEQ ID NO: 13**) linker domains are shown. The positively charged amino acids on one side of the helix have been circled.